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Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
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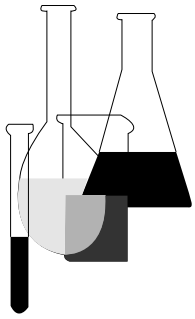
Health Effects Test Guidelines

OPPTS 870.8500
Toxicokinetic Test



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0132 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 870.8500 Toxicokinetic test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPPT 40 CFR 795.235 Toxicokinetic Test.

(b) **Purpose.** These studies are designed to determine the bioavailability of the test substance after dermal or oral treatment; ascertain whether the metabolites of the test substance are similar after dermal (assuming significant penetration) and oral administration; examine the effects of a multiple dosing regimen on the metabolism of the test substance after per os administration.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definition also applies to this test guideline.

Absorption toxicokinetics refers to the bioavailability, i.e. the rate and extent of absorption of the test substance, and metabolism and excretion rates of the test substance after absorption.

(d) **Test procedures**—(1) **Animal selection**—(i) **Species.** The rat is the animal species of choice since it has been used extensively for absorption, metabolism, and toxicological studies.

(ii) **Rat strain.** Adult male and female Fischer 344 rats should be used. At 7 to 9 weeks of age, the males should weigh 125 to 175 g and the females 110 to 150 g. The rats should be purchased from a reputable dealer and identified with ear tags upon arrival. The animals should be randomly selected for the testing groups, and no unhealthy animal is to be used for experimentation.

(iii) **Animal care.** (A) Animal care and housing should be in accordance with Department of Health, Education and Welfare Publication No. (NIH)–85–23, 1985. Guidelines for the Care and Use of Laboratory Animals, or its equivalent.

(B) The animals should be housed in environmentally controlled rooms with 10 to 15 air changes per hour. The rooms should be maintained at a temperature of 25 ± 2 °C and humidity of 50 ± 10 percent with a 12–h light/dark cycle per day. The rats should be kept in a quarantine facility for at least 7 days prior to use.

(C) During the acclimatization period, the rats should be housed in polycarbonate cages on hardwood chip bedding. All animals should be provided with certified feed and tap water ad libitum.

(iv) **Number of animals.** There should be at least four animals of each sex in each experimental group.

(2) **Administration of test substance**—(i) **Test substance.** Test substance of at least 99 percent purity, commercially available, should be used as the test substance. Since both nonradioactive and radioactive (uniformly ^{14}C-labeled) test substances are to be used, they should be chromatographed separately and analyzed together, to ascertain purity and identity. The use of ^{14}C-labeled test substance, diluted with unlabeled test substance, is required for all of the studies under this guideline, unless otherwise specified, as it will greatly increase the reliability and sensitivity of the quantitative assays and facilitate the identification of metabolites.

(ii) **Dosage and treatment.** (A) Two doses should be used in studies under this guideline, a low dose and a high dose. When administered orally, the high dose level should ideally induce some overt toxicity, such as weight loss. The low dose level should not induce observable effects attributable to the test substance. If feasible, the same high and low doses should be administered orally and dermally.

(B) Oral dosing should be accomplished by gavage after dissolving the test substance in a suitable vehicle. For dermal treatment, the doses should be administered in a suitable solvent and applied at a volume adequate to deliver the prescribed doses. The backs of the rats should be shaved with an electric clipper one day before treatment. The dose should be applied with a disposable micropipet on a specific area (2 cm^2 for rats) on the shaven skin. The dosed areas should be occluded with an aluminum foil patch secured in place with adhesive tape.

(iii) **Determination of test substance kinetics.** Each experimental group should contain at least four rats of each sex for a total of eight rats.

(A) **Oral studies.** (*1*) Group A should be dosed once orally with the low dose of the test substance.

(*2*) Group B should be dosed once orally with the high dose of the test substance.

(*3*) For the oral studies, the rats should be placed in individual metabolic cages to facilitate collection of urine and feces at 8, 24, 48, 72, and 96 h following administration. The cages should be cleaned at each time period to collect any metabolites that might adhere to the metabolic cages.

(B) **Dermal studies.** (*1*) Group C should be dosed once dermally with the low dose of test substance.

(*2*) Group D should be dosed once dermally with the high dose of test substance.

(*3*)(*i*) For the dermal studies, the test substance should be applied for 24 h. Immediately after application, each animal should be placed in a separate metabolic cage for excreta collection. At the time of removal of the aluminum foil, the occluded area should be washed with an appropriate solvent (see below), to remove any test substance that may be on the skin surface and the wash solvent assayed for the amount of test substance recovered. At the termination of the experiments, each animal should be sacrificed and the exposed skin area removed. The skin (or an appropriate section) should be solubilized and assayed for the test substance and its metabolites.

(*ii*) Before initiation of the dermal studies, an initial washing efficiency experiment should be conducted to assess the removal of the applied test substance by washing the exposed skin area with soap and water or organic solvents. Four rats, two of each sex, should be lightly anesthetized and then test substance applied to a specified area. After application (5 to 10 min), the areas should be washed with soap and water (two rats) or ethanol and water (two rats). The amount recovered should be determined to assess efficacy of test substance removal by washing of the skin.

(C) **Repeated dosing study Group E.** Four rats (two of each sex) should receive a series of single daily oral doses of nonradioactive test substance over a period of at least 14 days, followed at 24 h after the last dose by a single oral dose of ^{14}C-labeled test substance. Each dose should be at the low dose level.

(3) **Observation of animals**—(i) **Bioavailability**—(A) **Blood levels.** The levels of ^{14}C should be determined in whole blood, blood plasma, or blood serum at appropriate intervals from 1 to 96 h after dosing rats in Groups A through E. Four rats (two of each sex) of each group should be used for this purpose.

(B) **Urinary and fecal excretion.** The quantities of ^{14}C excreted in the urine and feces by rats in Groups A through E should be determined at 8 h, 24 h, 48 h, 72 h, and 96 h after dosing, and if necessary, daily thereafter until at least 90 percent of the applied dose has been excreted or until 7 days after dosing (whichever occurs first). Four animals (two of each sex) should be used for these analyses.

(ii) **Biotransformation after oral and dermal dosing.** Appropriate qualitative and quantitative methods should be used to assay the test substance and metabolites in the urine and fecal specimens collected from rat Groups A through D.

(iii) **Changes in biotransformation.** Appropriate qualitative and quantitative assay methodology should be used to compare the composition of ^{14}C-labeled compounds in excreta collected at 14 and 48 h after dosing

rat Group A with those in the excreta collected at 24 and 48 h after the ^{14}C-labeled test substance dose in the repeated dose study (Group E).

(e) **Data and reporting**—(1) **Treatment of results.** Data should be presented in tabular form.

(2) **Evaluation of results.** All observed results, quantitative or incidental, should be evaluated by an appropriate statistical method.

(3) **Test report.** In addition to the reporting requirements specified in the 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Species and strains of laboratory animals.

(ii) Information on the degree (i.e. specific activity for a radiolabel) and sites of labeling of the test substance;

(iii) A full description of the sensitivity and precision of all procedures used to produce the data.

(iv) Percent absorption by oral and dermal routes of rats administered ^{14}C-test substance.

(v) Quantity of isotope, together with percent recovery of administered dose in feces, urine, blood, and skin and skin washings (dermal study only for last two portions).

(vi) Quantity and distribution of ^{14}C-labeled test substance in various tissues, including bone, brain, fat, gonads, heart, kidney, liver, lung, muscle, spleen, and residual carcass.

(vii) Counting efficacy data should be made available to the Agency upon request.